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Review on Rapid (Pen Side) Tests, Their Applications and Drawbacks

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Abstract: Molecular diagnostics has become a growing part of the clinical laboratory. It includes all tests and methods to identify a disease and understand the predisposition for a disease analyzing DNA or RNA of an organism. The basic application of molecular diagnostic tests are to determine changes in sequence or expression levels in crucial genes involved in disease diagnosis. The scope of molecular diagnostics in molecular medicine could be expanded well beyond current nucleic acid testing. The aims of this review was to highlight rapid tests, their applications and some drawbacks in both molecular and serological levels. Pen-side diagnostic testing is often required to provide real time information about the health status of an animal and humans. The rapid results are generally needed in the face of a disease outbreak, where the diagnostician/clinician is presented with dead or dying individuals. Another use of pen-side tests is as part of a preventive medicine. Rapid tests are rapid and can be easy and some of them can be done at the field level without the need for special training for implementation and evaluation. Pen-side diagnostic testing is often required to provide real time information about the health status of an animal and humans. The rapid results are generally needed in the face of a disease outbreak, where the diagnostician is presented with dead or dying individuals. Hence , due consideration should be given to fulfill laboratory reagents and chemicals to do rapid tests in different circumstances and strategic screening tests should be done at different levels to develop skill of pen-side tests.

Key words: Pen-Side Tests · Molecular Diagnostics · Biosensor · DNA · RNA

INTRODUCTION

Molecular diagnostics has become a growing part of the clinical laboratory. It includes all tests and methods to identify a disease and understand the predisposition for a disease analyzing DNA or RNA of an organism. Rapid advances in molecular diagnostics enable basic research and results in practical diagnostic tests. The basic application is to determine changes in sequence or expression levels in crucial genes involved in disease. The scope of molecular diagnostics in molecular medicine could be expanded well beyond current nucleic acid testing. It plays an important role in practice of medicine, public health, pharmaceutical industry, forensics and biological warfare and drug discovery. Affordable point-of-testing diagnostic technology applied to resource-limited sites is one of the most promising biotechnologies for improving global health [1].

Basically, it is a simple to use diagnostic device used to confirm the presence or absence of a target analyte, such as pathogens or biomarkers in humans or animals, or contaminants in water supplies, foodstuffs, or animal feeds. The development of new diagnostics and their successful adoption in the field by end users requires consideration of various diverse factors including, but not limited to, scientific challenges, economic restrictions and practical considerations. High impact technology will allow sensitive and specific detection, it will be low cost and portable to ensure accessibility and it will have a user-friendly design that does not rely on sophisticated equipment [1].

Pen-side diagnostic testing is often required to provide real time information about the health status of an animal and humans. The rapid results are generally needed in the face of a disease outbreak, where the diagnostician/clinician is presented with dead or dying

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individuals. Another use of pen-side tests is as part of a preventive medicine. These test systems allow for the rapid, sensitive and specific detection of target antigen or antibody. Immunochromatography, a simplified assay system, has found widespread application as a field or pen-side test. In the future, DNA based diagnostics will become more adaptable for use in the field [2]. Therefore, the objective of this paper is to review on rapid tests, their applications and some drawbacks

Biosensor Test: A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction. Biosensors are employed in applications such as disease monitoring, drug discovery and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine, saliva, sweat). Typically, biosensors integrate biomolecular components (antibody, enzyme, nucleic acid etc.) and a physicochemical transducer, which generates a measurable signal, to enable detection of the biorecognition event (e.g. antibody-antigen interaction). The specificity of an immunochemical biosensor is dictated by the choice of antibody to be immobilised on the sensing surface. Biosensors show particular promise in the areas of clinical diagnostics, food analysis, bioprocessing and environmental monitoring and can be categorised as optical, mass, electrochemical and thermal devices [3].

Principles: The sample is allowed to pass through a membrane so that selection may be exercised and the interfering molecules are retained outside the membrane. The sample then interacts with the biological sensor and forms a product, which may be an electric current/charge, heat, gas or a suitable chemical. Principle of Biosensors consist of a biological component for sensing the presence and concentration of a substance and a transducer device.

Characteristics of Biosensors

Selectivity: Selectivity is perhaps the most important feature of a biosensor. Selectivity is the ability of a bioreceptor to detect a specific analyte in a sample containing other admixtures and contaminants. The best example of selectivity is depicted by the interaction of an antigen with the antibody. Classically, antibodies act as bioreceptors and are immobilised on the surface of the transducer. A solution (usually a buffer containing salts) containing the antigen is then exposed to the transducer where antibodies interact only with the antigens.

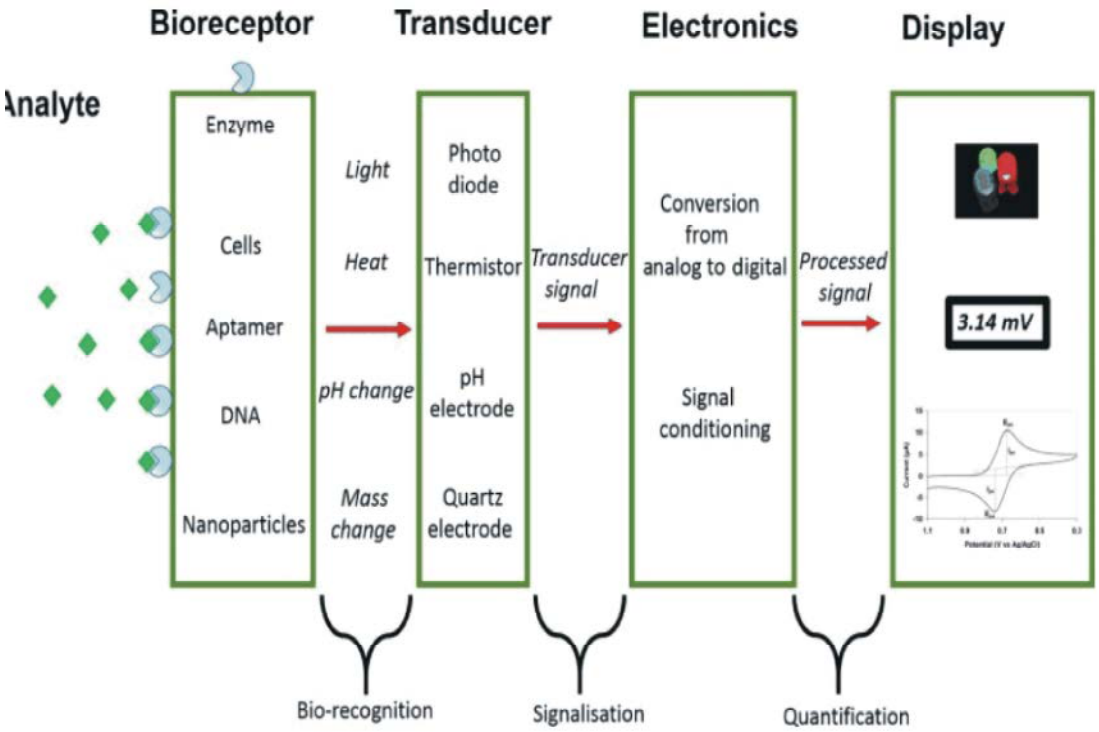

Fig. 1: Schematic Representation Biosensor

Reproducibility: Reproducibility is the ability of the biosensor to generate identical responses for a duplicated experimental set-up. The reproducibility is characterised by the precision and accuracy of the transducer and electronics in a biosensor. Precision is the ability of the sensor to provide alike results every time a sample is measured and accuracy indicates the sensor's capacity to provide a mean value close to the true value when a sample is measured more than once.

Stability: Stability is the degree of susceptibility to ambient disturbances in and around the biosensing system. Stability is the most crucial feature in applications where a biosensor requires long incubation steps or continuous monitoring.

Sensitivity: The minimum amount of analyte that can be detected by a biosensor defines its limit of detection (LOD) or sensitivity. In a number of medical and environmental monitoring applications, a biosensor is required to detect analyte concentration to confirm the presence of traces of analytes in a sample.

Linearity: Linearity is the attribute that shows the accuracy of the measured response (for a set of measurements with different concentrations of analyte) to a straight line, mathematically represented as- y=mc, where,

c is the concentration of the analyte,
y is the output signal and
m is the sensitivity of the biosensor.

Types of Biosensors: Biosensors started in the 1960s by the pioneers Clark and Lyons. Various types of biosensors being used are enzyme-based, tissue-based, immunosensors, DNA biosensors and thermal and piezoelectric biosensors.

Enzyme-Based Biosensors: The first enzyme-based sensor was reported by Updike and Hicks in 1967. Enzyme biosensors have been devised on immobilization methods, i.e. adsorption of enzymes by van der Waals forces, ionic bonding or covalent bonding. The commonly used enzymes for this purpose are oxidoreductases, polyphenol oxidases, peroxidases and aminooxidases [4].

Tissue-Based Biosensors: The first microbe-based or cell-based sensor was actualized by Diviès. The tissues for tissue-based sensors arise from plant and animal sources. The analyte of interest can be an inhibitor or a substrate of these processes. Rechnitz[9] developed the first tissue based sensor for the determination of amino acid arginine. Organelle-based sensors were made using membranes, chloroplasts, mitochondria and microsomes. However, for this type of biosensor, the stability was high, but the detection time was longer and the specificity was reduced [5].

Immunosensors and DNA Biosensors: Immunosensors were established on the fact that antibodies have high affinity towards their respective antigens, i.e. the antibodies specifically bind to pathogens or toxins, or interact with components of the host's immune system. The DNA biosensors were devised on the property that single-strand nucleic acid molecule is able to recognize and bind to its complementary strand in a sample. The interaction is due to the formation of stable hydrogen bonds between the two nucleic acid strands [6].

Magnetic and Piezoelectric Biosensors: Miniaturized biosensors detecting magnetic micro- and nanoparticles in microfluidic channels using the magnetoresistance effect have great potential in terms of sensitivity and size [7]. Piezoelectric Biosensors are of two types: the quartz crystal microbalance and the surface acoustic wave device. They are based on the measurement of changes in resonance frequency of a piezoelectric crystal due to mass changes on the crystal structure. Optical biosensors consist of a light source, as well as numerous optical components to generate a light beam with specific characteristics and to beeline this light to a modulating agent, a modified sensing head along with a photodetector [8].

Advantage and Disadvantage
Advantage:

- They can measure nonpolar molecules that do not respond to most measurement devices.
- They allow rapid continuous control.
- Speed of response (typically less than a minute) and ease of use is the main advantages offered by biosensors.
- Typically the smaller the device, the faster and more sensitive is the response.
- Biosensors can easily detect analytes in the micromolar to nanomolar range.
- Biosensors can serve exceptionally well in emergency.
- Situations or for on-site field applications.

Disadvantage:

- Heat sterilization is not possible as this would denature the biological part of the biosensor.
- The membrane that separates the reactor media from the immobilized cells of the sensor can become fouled by deposits.
- The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane.
- Changes in the reactor broth (i.e., pH) can put chemical and mechanical stress on the biosensor that might eventually impair it.

Application of Biosensors: Biosensors have seen a wide variety of applications, primarily in three major areas: biological monitoring, biomedical diagnostics and environmental sensing applications. There are many potential applications of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element and the potential for disposable portable detection systems to be preferred to sensitive laboratory based techniques in some situations [9].

In Medical Field: In the discipline of medical science, the applications of biosensors are growing rapidly. Glucose biosensors are widely used in clinical applications for diagnosis of diabetes mellitus, which requires precise control over blood-glucose levels. Biosensors are being used pervasively in the medical field to diagnose infectious diseases [10].

In medical applications biosensors are categorized as an *in vitro* systems. In which its measurements takes place in a test tube, a culture dish, a microtiter plate or elsewhere outside a living organism.. An example of an *in vitro* biosensor is an enzyme-conductimetric biosensor for blood glucose monitoring. There is a challenge to create a biosensor that operates by the principle of point-of-care testing, i.e. at the location where the test is needed [2].

The various other biosensors applications in medical include: quantitative measurement of cardiac markers in undiluted serum, microfluidic impedance assay for controlling endothelin-induced cardiac hypertrophy, immunosensor array for clinical immunophenotyping of acute leukemias, effect of oxazaborolidines on immobilized fructosyltransferase in dental diseases; histone deacylase (HDAC) inhibitor assay from resonance energy transfer, biochip for a quick and accurate detection of multiple cancer markers and neurochemical detection by diamond microneedle electrodes [11].

In biotechnology, analysis of the chemical composition of cultivation broth can be conducted in-line, on-line, at-line and off-line. As outlined by the US Food and Drug Administration (FDA) the sample is not removed from the process stream for in-line sensors, while it is diverted from the manufacturing process for on-line measurements. For at-line sensors the sample may be removed and analyzed in close proximity to the process stream. An example of the latter is the monitoring of lactose in a dairy processing plant. Off-line biosensors compare to bioanalytical techniques that are not operating in the field, but in the laboratory [12].

Drawbacks / Limitation of Biosensors:

- The first is the instability of the biological sensing component (enzyme, antibody, tissue, etc.), which can lose its activity in hours or days depending on the nature of the molecule and exposure to environmental stresses, such as pH, temperature or ionic strength.
- The second limitation is on the size of the physico-chemical transducers being used in biosensors

Lateral Flow Test: Rapid tests that don't need sophisticated instruments and trained man power and are value additions to the available tools under field conditions. Pen-side diagnostics in the form of immunochormatographic strip tests or Lateral Flow Device (LFD) have been increasingly adapted as diagnostic tools in human and veterinary medicine. These tests are suitable for either antigen or antibody detection. The various components of a LFIA have the capacity to transport fluid, including blood or serum, under capillary action and thus no external pumps are required [13].

Lateral flow or Lateral Flow Immunoassay (LFIA) technology is the most simple and successful rapid diagnostic testing platform derived from the latex agglutination test developed by Singer and Plotz [13]. The lateral flow tests offer greater access, affordability and convenience with wider acceptability for rapid diagnosis. Lateral flow test also known as, lateral flow test (LFT), lateral flow device (LFD), Lateral flow assay (LFA), Lateral flow immunoassay (LFIA), Lateral flow immunochromatographic assays, Dipstick, Pen-side test, Quick test, Rapid test and Test strip [14].

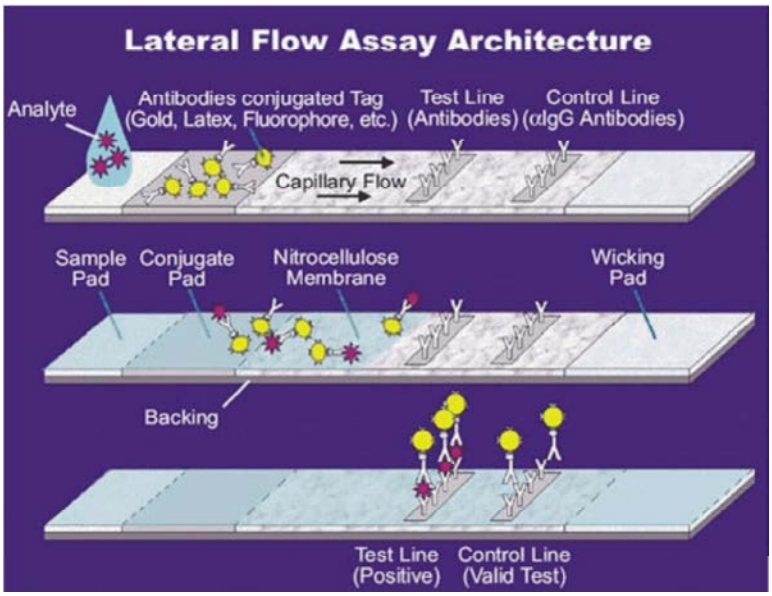

Fig. 2: An Illustration Of Lateral Flow Assay
Source: U.S. National Aeronautics and Space Administration

Principle of a Lateral Flow Assay: This simple technique is intended to detect the presence (or absence) of a target analyte in a sample (matrix). The technique is based on the use of different colored latex microspheres which are covalently linked to a control protein and to the corresponding antigen/antibody. The visualization of the antigen-antibody reaction is based on migration of the reagents by capillarity through a porous membrane. In the case of a positive sample a colored line (T line) will appear under the detection zone of the strip as the result of the formation of complexes between the detection reagent and the capture reagent. In the case of a negative sample the appearance of a colored line in the control area (C line) confirms the proper.

Detection System: The majority of the lateral flow assays use visual detection. The incorporation of gold nanoparticles or latex beads as labels allows visual examination of colours at the test and control lines, resulting in qualitative or semi-quantitative analysis. The use of colloidal gold, paramagnetic particles and fluorescent dyes as labels in lateral flow formats has opened up new horizons for LFIA technology by allowing true quantitative testing. The development of quantitative assays in lateral flow formats requires an additional fluorescence or optical strip reader which allows measurement of the intensity produced at the test and control lines of the strip The readers are based on the use of different wavelengths of light for illumination, in combination with a camera or charge-coupled device (CCD) imaging technology which allows capture of images of test and control lines [15, 16].

Advantage of Lateral Flow Test: Lateral flow immunoassays represent a well-established and very appropriate technology when applied to a wide variety of point-of-care (POC) or field use applications. The advantages of the lateral flow immunoassay system (LFIA) are well known:

- Established mature technology
- It does not require cold chain for transport or storage of the kit and results can be read with naked eyes without the need of specialized, costly instruments and trained personnel.
- Relative ease of manufacture equipment and processes already developed and available
- Easily scalable to high-volume production
- Stable shelf-lives of 12–24 months often without refrigeration
- Ease of use: minimal operator-dependent steps and interpretation
- Can handle small volumes of multiple sample types
- Can be integrated with onboard electronics, reader systems and information Systems
- Can have high sensitivity, specificity, good stability and rapid
- Relatively low cost and short timeline for development and approval

- Market presence and acceptance minimal education required for users and regulators

Applications: The application of Lateral flow has extended to other clinical conditions including infectious diseases.

- Therapeutic Monitoring
- Medical Diagnostic
- Animal Health
- Blood Banking
- Therapeutic Monitoring
- Food Safety
- Environmental quality
- Consumer Diagnostic
- Forensic Science

Drawbacks /Limitations: Lateral flow immunoassays, however, have also suffered from performance limitations, most notably sensitivity and reproducibility.

- Miniaturization of sample volume requirements below microliter level
- Multiplexing: simultaneous analysis of multiple markers difficult
- ntegration with onboard electronics and built-in QC functions challenging
- Sensitivity issues in some systems
- Test-to-test reproducibility challenging – limits applications in quantitative Systems

One-Step Elisa for Antigen Detection: The one step ELISA for detection of an antigen in a biological sample is based on a double sandwich assay in which two different antibodies against the antigen are used, a capture antibody labeled with biotin and a detection peroxidase conjugated antibody. Streptavidin coated plates are used as the support to which the sample and a master mix containing both antibodies is added. In the reaction the biotinylated antibody will bind to the streptavidin immobilized on the surface of the plate, the antigen in the sample will then be captured by the biotinylated antibody and the captured antigen will be then detected by the peroxidase conjugated antibody.

Advantage:

- Simplicity.
- Rapid.
- User friendly.
- Cost-effective

Drawbacks:

- Less sensitivity than PCR and RT-PCR.

Low Density Microarrays: Microarray technology allows highly parallel visualization of specific bindings in a rapid and low-sample volume format. Therefore, it plays a key role in many applications as diagnosis of diseases by Antibody-Antigen specific interaction. The low density microarray constitutes an important tool for either circulating Antibody detection or Antigen/Pathogen detection. Molecules in a microarray are attached to Super Epoxy glass slides. Primary amines on the molecules surface act as nucleophiles, attacking epoxy groups and coupling the molecule covalently to the surface. The visualization of the Antibody-Antigen reaction is based either on fluorescence or colorimetry.

Advantage:

- Multiplex
- Sensitivity
- Reliability
- Low sample volume required

Application:

- Detection of antigens and/or whole pathogens in a samples

Drawbacks:

- Printing and fluorescent technology are expensive

Agglutination: Agglutination is the clumping of particles. The word agglutination comes from the Latin *agglutinare*, meaning:- glueing. Agglutination is the process that occurs if an antigen is mixed with its corresponding antibody called isoagglutinin, which is commonly used in blood grouping. Depending on the nature of antigens, the agglutination reaction can be active or passive. The agglutination is said to be active when the surface antigens participating in the reaction are natural parts of the particle. The agglutination of RBCs for blood grouping and agglutination of bacterial cells for serotyping are examples of active agglutination. Passive agglutination is when the soluble antigens are coated on the surface of carrier particles. Agglutination tests detect antibody or antigen and involve agglutination of bacteria, red cells, or antigen- or antibody-coated latex particles.

They rely on the bivalent nature of antibodies, which can cross-link particulate antigens. Serial dilutions of serum are tested for their ability to cause or inhibit agglutination and the highest dilution that causes or inhibits agglutination is reported as the antibody or antigen titer. IgM causes agglutination more effectively than IgG [17].

Card Agglutination: The advantages of the CAT are that, it is sensitive, may be undertaken either in the laboratory or in the field and gives a result within a few minutes. The card agglutination test for trypanosomiasis, or CATT is applied in *gambiense* for screening of the population at risk. On a test card, the reagent, consisting of stained trypanosomes, is mixed with blood. The test uses the formalin fixed variable antigen types of *T. evansi* that are used in the agglutination test. The test, which is simple to perform, has been used for diagnosis of *T. evansi* and also used to detect antibodies against *T. evansi*. The blue agglutination indicates the presence of trypanosome-specific antibodies. To increase specificity, the test is often repeated on dilutions of serum or plasma. No similar reagent for serodiagnostic of *T. b. rhodesiense* infection exists [18].

Latex: Latex agglutination test has been the method of choice in the development of a rapid test kit in many fields including clinical and veterinary medicines. In principle, latex agglutination test is based on agglutination reactions between antigen and antibody. Latex beads are used as a solid support for the antibody (antigen) to be adsorbed onto them. The latex beads with adsorbed antibody (antigen) are used to detect antigen (antibody) present in biological samples. Polystyrene latex beads are commonly used in the latex agglutination test because of their strong hydrophobic characteristic that is ideal for the adsorption of materials such as proteins by a simple passive adsorption [19].

Latex tests have been developed to detect specific infectious diseases, autoimmune diseases, hormones, drugs and serum proteins. The original method for attachment of proteins (antibody) to latex beads was passive adsorption. Covalent coupling method using functionalized latex beads such as carboxylated polystyrene latex beads can produce more stabilized protein-latex complex compared to passive adsorption method. It has then become a method of choice in the development of latex tests such as for the detection of anti-cysticercus antibodies, IgM quantification in cerebrospinal fluid , and detection of avian influenza virus subtype H5N1. Although covalent coupling methods have many advantages, the passive adsorption method is still widely used until today because of its simplicity and flexibility [20].

Advantage:

- Ease of performance.
- Speed of performance, usually requiring few minutes.
- High degree of sensitivity
- Cheap, quick and practical serologic test
- It is a highly sensitive and specific test when applied on undiluted whole blood .

Applications:

- Suitable for diagnosis of trypanosomosis
- Identification of an organism with known antibodies
- Identification of serum antibodies with known antigens.
- Several antibodies can be detected
- The test is used For Mass Screening Of Populations In Whole Blood For Detection Of Hemoparasities

CONCLUTIONS AND RECOMMENDATIONS

Rapid tests are rapid and can be easy and some of them can be done at the field level without the need for special training for implementation and evaluation. Pen-side diagnostic testing is often required to provide real time information about the health status of an animal and humans. The rapid results are generally needed in the face of a disease outbreak, where the diagnostician is presented with dead or dying individuals.

From this paper, the following recommendations were forwarded:

- Due consideration should be given to fulfill laboratory reagents and chemicals to do rapid tests in different circumstances.
- Strategic screening tests should be done at different level to develop our skill and experience on these rapid tests.
- Attempt should be made to expand government and Private laboratory Services with enough laboratory equipments.

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